In vitro bulk/surface erosion pattern of PLGA implant in physiological conditions: a study based on auxiliary microsphere systems

Sonia Boimvaser,[a] Nicolás R. Mariano,[a] Ludmila N. Turino,[a] Jorge R. Vega[b,*]

[a] Laboratorio de Química Fina. Instituto de Desarrollo Tecnológico para la Industria Química (INTEC). Universidad Nacional del Litoral (U.N.L.). Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Ruta Nacional 168, Km 472, 3000 Santa Fe, Argentina.

[b] Grupo de Polímeros y Reactores de Polimerización. Instituto de Desarrollo Tecnológico para la Industria Química (INTEC). Universidad Nacional del Litoral (U.N.L.). Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Ruta Nacional 168, Km 472, 3000 Santa Fe, Argentina.

* To whom all correspondence should be addressed. Tel: +54-342-4511593; fax: +54-342-4511079. *E-mail address*: soniaboimvaser@gmail.com

---

E-mail addresses:

N. Mariano: nmariano@santafe-conicet.gov.ar

L. Turino: lturino@intec.unl.edu.ar

J. Vega: jvega@santafe-conicet.gov.ar

Abstract

This work investigates the degradation of PLGA implants in an aqueous medium maintained at physiological pH ≈7.4. Two limiting systems are also investigated, which involve the degradation of PLGA microspheres in two different media characterized by: i) a non-regulated pH, for emulating the autocatalyzed degradation in the implant core; and ii) a regulated physiological pH, for emulating the uncatalyzed degradation at the implant surface. The degradation experiments were carried out along 40 - 50 days, and samples withdrawn during this period were characterized by gravimetry, electronic microscopy, and size exclusion chromatography. Experimental results suggest that PLGA implants are degraded according to a time-variant spatial pattern, which depends on the pH of the surrounding medium. Initially, the implants suffered a typically bulk erosion process, governed by the acidification of the implant core; and after breakage of the implant wall, the regulated physiological pH induces a surface erosion process. The two auxiliary microspheres-based experiments were useful to elucidate the degradation phenomena occurring in the PLGA implants. The evolution of the mass loss and the weight-average molecular weight along the degradation can be successfully predicted by simple mathematical models based on first-order kinetics.

Keywords: PLGA, implant, microspheres, non-homogeneous degradation, bulk/ surface erosion, physiological condition

1 Introduction

Poly(D,L-lactide-co-glycolide) (PLGA) is one of the few biocompatible and biodegradable polymers approved by U.S. Food and Drug Administration for its use in pharmaceutical products or biomedical devices. It has been successfully used for designing drug delivery systems such as scaffolds, microspheres, nanospheres, and implants [1,2]. Along the last two decades, special efforts have been done in the development of in situ forming devices (implants and microspheres) as alternative delivery systems. These systems are injected into the body and once in contact with the physiological fluids, solidify to form a semi-solid depot able to entrap a drug. Some of their advantages are: ease of fabrication, less invasive administration, and less stressful manufacturing conditions for sensitive bioactive molecules [3,4]. The release profile of the entrapped drug depends on its diffusion through the polymer matrix and/or on the matrix degradation/erosion processes [5,2]. For this reason, the degradation of PLGA is an important issue to be considered in controlled drug release systems [6].

The erosion of degradable polymers starts with degradation, i.e. the process of chain scission. For biodegradable polymers, chain scission occurs by hydrolysis of the functional groups in an aqueous environment. Then, degradation products such as oligomers and monomers are released from the polymer matrix to the surrounding medium leading to the characteristic mass loss of the erosion process. Hydrolysis starts with the penetration of water into the polymer matrix. When hydrolysis is slow compared to diffusion of water inside the matrix, then the whole polymer matrix is simultaneously eroded. This behavior is known as bulk erosion. In contrast, when hydrolysis is faster than the water uptake, then erosion mainly occurs at the matrix surface. This degradation process is termed as surface erosion. Nevertheless, these two erosion mechanisms are ideal limiting cases that cannot be unambiguously assigned to most polymer degradation processes [7].

It is generally accepted that a PLGA matrix in an aqueous medium suffers bulk erosion [8,9]. However, some authors have found that PLGA sponges also suffer surface erosion [10]. The bulk degradation of PLGA can be non-homogeneous or homogeneous, depending on the size of the studied system. In large PLGA devices, the degradation is normally non-homogeneous and becomes faster in the core than in the surface. Such behavior is a consequence of an internal autocatalytic effect induced by the acidic degradation products that cannot easily diffuse outside the matrix [6]. Such non-homogeneous degradation process can be identified by a bimodal molecular weight distribution (MWD), where the low molecular weight (MW) mode corresponds to the shorter polymer chains present in the core, and the high MW mode corresponds to the longer polymer chains present in the outer region of the matrix [11,8]. In contrast, small PLGA devices (e.g., microspheres with a mean diameter lesser than 10 μm) typically suffer a homogeneous degradation, because they do not present meaningful diffusional problems and therefore no accumulation of soluble oligomers or monomers occurs in the matrix. Thus, a homogeneous degradation process can be identified by an unimodal MWD [12].

In order to mimic physiological conditions, PLGA degradation studies are normally carried out in a pH 7.4 phosphate buffer saline media [13-17,10]. However, the typical release of acidic products produced by the hydrolysis process, tends to diminish the pH of the medium along the time [6], and therefore strict physiological conditions are not properly reproduced. Generally, no rigorous monitoring and/or control of the pH are reported while studying in vitro PLGA degradation processes.

This work aims at investigating the degradation process undergone by in situ forming PLGA implants in an aqueous medium at a maintained pH 7.4, and 37 ºC, in order to simulate physiological conditions. For a better understanding of the degradation phenomenon occurring in the core and at the surface of the polymer matrix, two additional limiting systems were designed and investigated: 1) the degradation of microspheres in an aqueous medium with a non-regulated pH (for simulating the autocatalytic hydrolysis inside of the polymer matrix); and 2) the degradation of microspheres in an aqueous medium with a maintained pH 7.4 (for simulating the uncatalyzed degradation at the polymer surface). Gravimetric analysis, scanning electron microscopy, and size exclusion chromatography (SEC) were used to identify the degradation mechanism. In all experiments, the SEC chromatograms, the weight-average MW (M_w), and the remaining mass of the polymeric systems were used as quantitative measures of the degradation/erosion processes along the time, and served to propose a simple model of the degradation mechanism.

2 Materials and Methods

2.1 Materials

The following chemicals (reagent grade) were used: dimethylsulfoxide and ethyl acetate, from Sigma Aldrich (Argentine), and tetrahydrofurane (THF), dichloromethane, polyvinyl alcohol, sodium hydroxide, sodium chloride, sodium phosphate, and sodium azide (NaN_3), from local suppliers. Dialysis tubing cellulose membranes (MW cut-off 12.4 kDa, average flat width 0.4 in., Sigma, from Sigma Aldrich, Argentine) were used for the in vitro assays. Distilled and deionized (dd) water was used to prepare all the solutions. PLGA of copolymer ratio 50:50 (RG 502H, from Boehringer Ingelheim, Germany) was used in all experiments. Based on the SEC measurements, an absolute M_w = 6900 g/mol was determined. More details on the SEC data processing procedure are given in section 2.5.

2.2 Degradation media

The degradation studies were carried out in two different media: 1) a pH 7.4 phosphate buffer saline (PBS) solution, which provides physiological ionic strength and buffered pH, and 2) a saline solution (SS), which provides physiological ionic strength without buffering effect. The PBS medium was prepared by dissolving 8.77 g of NaCl, 11.5 g of Na_2HPO_4, 2.28 g of NaH_2PO_4, and 0.2 g of NaN_3 in 1 L of dd-water. The pH was then adjusted to 7.4 by using NaOH. The SS medium was prepared by dissolving 8.77 g of NaCl and 0.2 g of NaN_3 in 1 L of dd-water. The pH of the initial solution was 6.4.

2.3 In situ forming implants: preparation and degradation study

The polymer solution was prepared in a glass flask by mixing 0.4 g of PLGA and 1 mL of dimethylsulfoxide until complete dissolution. PLGA implants were formed in situ by phase inversion [18], as first introduced by Dunn [19]. This precipitation process consists in the injection into an aqueous medium of a water insoluble polymer like PLGA dissolved in a water soluble solvent. The solvent diffuses out of the polymer while water diffuses into the polymer matrix. The polymer solidifies in contact with water, thus forming a solid polymeric implant. Several dialysis bags were filled with a polymer solution that contained an initial PLGA mass of 75 mg (M_0). Then, the bags were placed into glass flasks containing 10 mL PBS for the in situ implant formation.

Degradation assays of PLGA implants in the PBS medium with regulated pH (Imp-PBS) were performed at 37 °C on a horizontal shaker at 50 rpm. The pH of the medium was measured every day. When the pH dropped to 7, the implants were washed with dd-water, and the medium was replaced with fresh PBS, in order to keep the pH of the environment close to 7.4. During 50 days, implants were withdrawn at predetermined time intervals, washed with dd-water, and vacuum dried to constant weight. Along the degradation time (t), the residual mass of each implant, $M(t)$, was gravimetrically determined; and the percentage remaining mass, $RM(t)$, was calculated as follows:

$$RM(t)\ [\%] = M(t)/M_0 \cdot 100 \quad (1)$$

Dry implants were stored at -20 °C for further analyses. The study was conducted by duplicate.

2.4 Microsphere systems: preparation and degradation study

Microspheres were prepared using an oil-in-water emulsion solvent extraction method, adapted from Sah [20]. The emulsion was prepared by adding 5 mL 20% w/v PLGA solution in ethyl acetate (oil phase) into 15 mL 2% w/v aqueous polyvinyl alcohol solution (water phase). During the addition, the aqueous phase was stirred at 4000 rpm (Ika® T-25 Ultra-Turrax® Digital Homogenizer with an Ika® Dispersing Tool S25N-18G, Cole Parmer, Germany). After 10 minutes of homogenization, 70 mL of dd-water were added to the emulsion and stirred during 60 minutes with a magnetic mixer. The emulsion was centrifuged at 2750 rpm and the pellet was vacuum dried at liquid nitrogen temperature until constant weight. Then, two microsphere systems were prepared by adding 65 mg (M_0) of microspheres into glass flasks containing 10 mL of either PBS (Ms-PBS system) or SS (Ms-SS system).

All degradation assays were performed at 37 °C on a horizontal shaker at 50 rpm. The pH of the medium was measured every day. Only in the Ms-PBS system, the pH was maintained at 7.4, by following the same strategy mentioned in section 2.3 for Imp-PBS. Along 50 days (for Ms-PBS) and 40 days (for Ms-SS), samples were withdrawn at predetermined time intervals, centrifuged, washed with dd-water, and vacuum dried until constant weight. Then, $M(t)$ was gravimetrically determined and $RM(t)$ was calculated through Eq. (1). Dry microspheres were stored at -20 °C for further analyses. The study was conducted by duplicate.

2.5 Molecular characterization by SEC

Single-detection SEC was used for the molecular characterization of the samples. The chromatograph (Waters Corp., pump model 1525, automatic injector model 717 plus) was fitted with a set of 4 fractionation columns (Waters Styragel, HR 0.5, HR 1, HR 2, HR 3; 7.8 mm × 300 mm, 5 μm), and a differential refractive index detector (Waters, model 2414). The mobile phase was THF at 1.0 mL/min and room temperature. For all analyzed samples, the injection volume was 200 μL. The raw chromatograms were acquired with the Breeze software (Waters Corp.), and then exported for a further processing.

The chromatographic system was initially calibrated with a set of 5 narrow polystyrene (PS) standards (Shodex SM-105, Showa Denko), in the MW range: 1200 g/mol to 55100 g/mol. The PS standards were prepared at a nominal concentration of 0.5 mg/mL. After injections of the standards, 5 pairs of points $\{M_{PS}, V_p\}$ were stored, where M_{PS} (g/mol) is the peak MW of each standard (as reported by the supplier), and V_p (mL) is the elution volume corresponding to the chromatogram maximum.

At each incubation time, samples of Ms-PBS, Ms-SS, and Imp-PBS were prepared at a nominal concentration of 2 mg/mL. To these effects, 2 mg of microspheres were directly dissolved in 1 mL of THF and then filtered. As implants are known to degrade non-homogeneously, they were first dissolved in dichloromethane to obtain a representative sample. Then, aliquots corresponding to approximately 2 mg of PLGA were vacuum-dried, dissolved in 1 mL of THF and filtered.

The estimation of reliable MWs by SEC presented two main difficulties. First, uncertainties in the chromatogram baselines at high elution volumes (V), originated by the THF peak that elutes close to the ample variety of PLGA oligomers typically present in a degraded sample. Fortunately, such uncertainties have no meaningful influence on the calculated M_w [21]. To systematize the baseline correction of all raw chromatograms, the upper-limit of the V-range was selected just before the appearance of the THF peak. Second, the lack of narrow PLGA standards prevented a direct calculation of absolute MWs of the samples. To overcome this problem, the universal calibration concept of SEC [22] was applied. To this effect, the peak MW of each PS standard, M_{PS}, was transformed into an equivalent PLGA MW, as follows [23]:

$$\log M_{PLGA} = \frac{1}{1+a_{PLGA}} \cdot \log \frac{K_{PS}}{K_{PLGA}} + \frac{1+a_{PS}}{1+a_{PLGA}} \cdot \log M_{PS} \qquad (2)$$

where $\{K_{PS}, a_{PS}\}$ and $\{K_{PLGA}, a_{PLGA}\}$ are the Mark-Houwink-Sakurada (M-H-S) constants, for PS and PLGA, respectively; and $\log M_{PLGA}$ (or $\log M_{PS}$) represents the decimal logarithm of M_{PLGA} [or M_{PS}]. For PS and PLGA in THF

as solvent, the following M-H-S constants were adopted [24,25]: K_{PS}=0.011 mL/g; a_{PS} = 0.725; K_{PLGA} = 0.0107 mL/g; and a_{PLGA} = 0.761.

After application of Eq. (2) onto the original M_{PS}, the resulting 5 pairs of points {logM_{PLGA}, V_p} were fitted by means of a third-order polynomial (R^2 = 0.9997), yielding the following PLGA-based MW calibration:

$$\log M_{PLGA}(V) = -0.0005133 \cdot V^3 + 0.04972 \cdot V^2 - 1.686 \cdot V + 22.61 \quad (3)$$

The MWDs of implants and microspheres along the degradation time were determined by combining the corresponding baseline-corrected chromatograms with Eq. (3). From the MWDs, the weight-average MWs were calculated.

2.6 Scanning electron microscopy

The structural morphologies of implants and microspheres were examined using scanning electron microscopy (SEM) with an acceleration voltage of 20 kV, in a JEOL JSM-35C equipped with the image acquisition program SemAfore (JEOL). Wet implants were freeze-fractured in liquid nitrogen, put over an aluminum stub, and subsequently lyophilized. Non-degraded microspheres were resuspended in dd-water and one drop of this suspension was put over an aluminum stub until water evaporation. All prepared samples were then sputter coated with gold under argon atmosphere, using soft conditions (two sputterings of 10 seconds each, with an intensity of 15 mA). Diameters of implant pores and microspheres were measured in the SEM photomicrographs with an image processing program (ImageJ 1.40g, National Institutes of Health).

2.7 Statistical analysis

For the analysis of microsphere diameters and implant pore sizes, data were reported as follows: mean ± standard deviation. Fit goodness was evaluated through the standard correlation coefficient (R^2) for linear regressions; and with the Chi-square test (significance level of 0.01) for non-linear regressions. Error bars of the experimental data points in the graphics represent the standard deviations.

3 Results

Figure 1 shows the SEM photomicrographs of an implant formed by phase inversion after 1 day of incubation in PBS and microspheres prepared by the method described in section 2.4. The polymer solution formed a continuous implant with pores in the entire matrix (**Figure 1a**). The average pore diameter of the implants was 39.3 ± 21.5 μm. **Figure 1b** shows microspheres with smooth and regular surfaces. The average particle diameter was 3.0 ± 0.7 μm.

For the three investigated systems, the pH in the degradation media was rigorously monitored in order to obtain information about the release of acids from the matrices (**Figure 2**). During the first 15 days of degradation, the pH of the non-regulated Ms-SS medium dropped to 2.7. Thenceforth, the pH of the medium suffered an additional drop to 2.4

at day 25, and then this value was almost constant up to the end of the assay (**Figure 2a**). The final pH 2.4 is probably due to the regulation by the lactic and glycolic acids that buffered the pH of the medium thus avoiding a further decrease. For Imp-PBS and Ms-PBS assays, the variations of pH along the assay are shown in **Figure 2b** and **Figure 2c**, respectively. In both figures, the abrupt pH increases show the fresh medium replacements required to maintain the pH within the range 7.0 – 7.4. Thus, the Ms-PBS system required only three medium replacements, at days 8, 22, and 43 (**Figure 2c**). In contrast, the implants required five medium replacements, at days 8, 14, 18, 25, and 36 (**Figure 2b**), being necessary a more frequent PBS replacement at day 14 and 18.

Photomicrographs of the implants were taken at four different degradation times (**Figure 3)**. After 3 days of incubation, an almost uniform porous matrix was observed. On day 12 of degradation, implants exhibited a collapsed core with an undamaged wall. Between days 12 and 22, the matrix suffered a noticeable breakage and lost its wall integrity. At day 33 of incubation, the implants continued their erosional processes and reached a more damaged structure; and after day 33, they became too fragile, which makes their use for SEM impossible.

The *RM* profiles of the three degradation systems are shown in **Figure 4**. Along the first five degradation days, no meaningful change in *RM* was detected in the Ms-SS samples (lag phase). From day 8 onwards, the remaining mass of microspheres decreased significantly; and after 40 days of degradation, only 4% of remaining mass was detected in Ms-SS. In contrast, Imp-PBS and Ms-PBS showed a mass reduction from the first day, reaching *RM* of 15% and 20%, respectively, after 50 days of incubation.

Some authors [26,27] suggested that the PLGA erosion typically follows a first order reaction kinetics. The evolution of the remaining mass is here adjusted by:

$$\ln[\,RM\,(t-t_{\mathrm{lag}})] = -k\,(t-t_{\mathrm{lag}}) + 4.605\,; \qquad (t \geq t_{\mathrm{lag}}) \tag{4}$$

where k is the kinetic exponential constant of erosion, and t_{lag} is the time corresponding to the lag phase. The term 4.605 = ln(100) accounts for the 100% of remaining mass existing for all $t<t_{lag}$. This simple model was able to discriminate the three degradation systems, which fairly followed a pseudo first order behavior (**Figure 5**). The Ms-SS data were fitted from day 8 onward (i.e., t_{lag} = 8 days). From Eq. (4), the calculated pseudo first-order kinetic constants as well as their corresponding R^2 values are presented in **Table 1**.

For the three investigated systems, the chromatograms were normalized to exhibit areas proportional to their corresponding *RM* (**Figure 6**). The chromatogram of the original PLGA presented a main mode and a small secondary mode, with their maxima placed at V≈22 mL and 30 mL (corresponding to logarithmic MWs given by $\log M_p$≈3.8 and 2.9, respectively). The presence of a secondary mode in the chromatogram of the raw polymer was also reported for

polylactide (PLA) [28] and for PLGA Resomer RG503H [29]. Despite the observed bimodality, our original PLGA has a M_w value within the range expected for the RG 502H polymer from Boehringer Ingelheim. **Figure 7** shows the evolutions of both maxima along the degradation time. The low MW mode of Ms-SS showed a slight shift towards higher values, while the high MW mode showed a rapid shift towards lower MWs. At day 22, low and high MW modes merged in a single one, thus producing a unimodal chromatogram centered at around logM_p≈3.0 (**Figure 6a** and **Figure 7a**). The high MW mode of Imp-PBS presented a shift to lower MW until reaching logM_p≈3.5 on day 12, while the low MW mode remained almost invariant at logM_p≈2.9 (**Figure 6b** and **Figure 7b**). Along the first days, the erosion process produced a more pronounced mass reduction in the high MW mode, while a relative increase was observed in the low MW mode. From day 12 onwards, the erosion continued and the chromatograms of Imp-PBS samples were similar (**Figure 6b**).

All the Ms-PBS chromatograms were similar along the degradation period (**Figure 6c**), exhibiting two modes at logM_p≈3.8 and 2.9, respectively (**Figure 7c**). As in the case of Imp-PBS, the low MW mode showed an increase in its relative mass along the first days of degradation.

For the three systems, the evolution of the measured M_w along the degradation time is shown in symbols in **Figure 8**. Although the Ms-SS mass loss was negligible during the first days, M_w immediately decreased after its contact with the aqueous medium until reaching an almost constant value of 1000 g/mol after day 14 of degradation. Some authors [15,30] have suggested that this MW value corresponds to PLGA oligomers that are dissolved in the medium (i.e., turning the hydrolysis into a dissolution process, and therefore these oligomers were absent in the analyzed SEC samples). As in the case of Ms-SS, the M_w of Imp-PBS showed a decrease up to day 15, which was followed by an almost constant M_w value of 3300 g/mol. Despite the data dispersion, Ms-PBS showed a rather constant M_w value during the 50 days of the study (**Figure 8**), which is consistent with the absence of chromatogram shifts (**Figure 6c**).

4 Discussion

Water uptake and degradation properties can be modified when the concentration and type of salt are involved in degradation studies of PLGA or PLA [31,32,28]. In the present work, the visual aspect of the microspheres degraded in the two media was different. While the Ms-SS had an opaque and white appearance, the Ms-PBS were translucent probably as a consequence of their greater capability of water uptake (water acts as a plasticizer that decreases the glass transition temperature of the polymer and clarifies the polymer mass [33]).

Hydrolysis of PLGA generates soluble products that decrease the pH of the medium [8,34]. In vivo, the continuous fluid exchange makes the pH of the physiological medium to be maintained at 7.4. In order to simulate physiological conditions, most studies are carried out in media of regulated pH 7.4. Some authors reported the maintenance of the pH of the medium through its total replacement with fresh medium once a week [13,14], every time a sample is withdrawn

[15,16], or every time they consider it as appropriate [17]. In this work, we have strictly monitored and maintained the physiological pH by replacing the medium every time it dropped to 7 in Ms-PBS and Imp-PBS.

Photomicrographs of the implants shown a noticeable breakage and lost of the wall integrity between days 12 and 22, which justify the important pH drop observed around days 14 and 18 (**Figure 2b**). The faster pH decrease may be attributed to the release of soluble hydrolytic products that were entrapped within the matrix.

The erosion profile observed for Ms-SS (i.e., a negligible mass loss in the first period followed by a rapid mass reduction) is typical of bulk eroding polymers [26]. The absence of a lag phase (t_{lag}=0) for the *RM* of both Imp-PBS and Ms-PBS is probably due to the release of soluble oligomers when the polymer was put in contact with the PBS medium. In the case of Imp-PBS, the oligomers could be dissolved in the solvent that diffused out of the polymer solution during the phase inversion process. In contrast, the soluble oligomers that were entrapped in Ms-PBS during their manufacturing process would be easily released as a consequence of the lack of diffusion problems in these small devices. Unlike Ms-PBS, the poor water uptake of Ms-SS could lead to a slower release of these oligomers from the polymer matrix, thus justifying the observed lag phase.

As expected, microspheres were eroded faster in the medium of non-regulated pH (autocatalyzed) than in the medium of maintained pH 7.4 (uncatalyzed). Implants had an intermediate degradation behavior (autocatalyzed in the core and uncatalyzed on the surface), and presented an erosional profile between the two microspheres profiles (**Figure 5**). The estimated kinetic erosion constants of Eq. (4) show a reasonable tendency: $k_{Ms\text{-}SS} > k_{Imp\text{-}PBS} > k_{Ms\text{-}PBS}$ (**Table 1**).

4.1 A global degradation model

This section aims at developing a simple model able to describe the implant degradation pattern, $M_w(t)$, in a isosmotic medium maintained at a physiological pH (Imp-PBS), as an intermediate case between the degradation profiles of Ms-SS and Ms-PBS. Since the original PLGA presented a bimodal chromatogram, it was impossible to unambiguously assign the low and high mode of the MWDs to the core and surface degradation respectively, as reported in the literature [11,8]. Instead, a chromatogram pattern associated with the pH of the surrounding medium is here presented. At the low pH caused by the degradation products (i.e., Ms-SS), the large polymer molecules are rapidly cleaved into smaller chains, thus shifting the high MW mode towards lower MWs (**Figure 6a**). In contrast, at physiological pH (i.e., Ms-PBS) the hydrolysis is slower, long polymer chains are present along the degradation period, and the chromatograms do not present important changes. Typically, a surface eroding polymer exhibits unchanged chromatograms along its degradation. This is because degradation gradually occurs at the polymer surface, and the generated low MW chains (oligomers) are released into the medium [35]. According to the chromatographic profiles of **Figure 6c**, the PLGA microspheres degraded in simulated physiological medium (Ms-PBS) undergoes a surface-like erosion process. This somehow surprising result may be due to our particular experimental conditions that involved small matrices and a strict pH control of the medium.

Therefore, the degradation process of the Imp-PBS can be modeled through two consecutive phases. The first one is governed by an autocatalytic process that occurs at decreasing pH in the implant core (resembling the Ms-SS pattern). The second phase consists in an uncatalyzed process that occurs after the breakage of the implant wall, in a medium maintained at a physiological pH (resembling the surface-like erosion pattern of Ms-PBS). The implant breakage was experimentally corroborated by the pH profile and the photomicrographs of Imp-PBS (**Figure 2b** and **3**).

Most publications report the degradation of PLGA bulk materials at pH 7.4 and 37 ºC to be dominated by an autocatalized bulk erosion occurring along the whole degradation period. However, a combined bulk / surface erosion mechanism has also been reported for porous scaffolds of PLGA of high MW, under weekly control of the pH of the medium [10]. In such work, the authors reported a degradation mechanism based on a first period of autocatalyzed bulk degradation, followed by a release of the accumulated acidic products and by a second period of surface degradation. In the present work, we have observed a similar degradation pattern for in situ forming PLGA implants that presents remarkable differences (e.g., formation system, co-polymer ratio, molecular weight, etc.). Additionally, we have found that PLGA surface degradation occurs when there is a strictly controlled physiological pH and in absence of acidic products accumulated in the surrounding medium. The PBS and SS degraded microspheres were useful for elucidating the PLGA degradation mechanism; and therefore we were able to associate the microsphere degradation medium with the two degradation periods observed in the Imp-PBS.

Based on the above observations as well as in the general trends exhibited by the experimental points of **Figure 8**, a simple mathematical model for describing the $M_w(t)$ profiles can be proposed. To this effect, note that $M_w(t)$ of both Imp-PBS and Ms-SS experiments exponentially decrease during the first days; and then remain almost constant along the degradation time. On the other hand, $M_w(t)$ of the Ms-PBS experiment can be considered as constant. In the case of Imp-PBS, it was seen that $M_w(t)$ decay during the autocatalyzed degradation period until the implant breakage, while remain almost constant during the uncatalyzed degradation period. Therefore, the following model can be written:

$$M_w(t) = \begin{cases} M_w(0)\, A\, e^{-\lambda t}; & t \le \mathrm{t_{ib}} \quad \text{(autocatalyzed degradation)} \\ M_w(0)\, A\, e^{-\lambda t_{\mathrm{ib}}}; & t > \mathrm{t_{ib}} \quad \text{(uncatalyzed degradation)} \end{cases} \tag{5}$$

where $M_w(0)$ is the initial weight-average molar mass of the sample; A and λ are parameters that must be determined from experimental data; and t_{ib} is the time corresponding to the implant breakage. **Figure 8** show the predictions of the model (see the adjusted parameters in **Table 2**). The resulting t_{ib} = 17.4 days was calculated from the intercept of the

autocatalyzed and uncatalyzed degradation curves. Notice that the model is also able to adequately describe the two limiting cases of Ms-SS and Ms-PBS. For Ms-PBS, $\lambda \approx 0$ and therefore the model directly predicts a constant $M_w(t)$.

Figure 9a shows a general scheme of the typical degradation processes (known as bulk erosion and surface erosion) and the proposed degradation process undergoes by Imp-PBS. As an intermediate case, Imp-PBS exhibits a combination of both degradation processes (see also **Figure 9b**). Initially, an acidic autocatalyzed process in the core governs the degradation until the implant breakage (at $t=t_{ib}$). During this period, the chromatograms presented a gradual shift to lower MWs, thus resulting in a decrease of the M_w values (autocatalyzed degradation phase). During the second phase of degradation (at $t>t_{ib}$), no significant accumulation of acidic products occurs in the implant, being the hydrolysis governed by a surface-like degradation process. Along this phase, chromatograms remained almost unchanged (uncatalyzed degradation phase), leading to constant M_w values. **Figure 9b** summarizes the two-steps degradation process of an implant maintained at a simulated physiological medium (Imp-PBS).

5 Conclusions

This work elucidates some aspects of the degradation process that in situ formed PLGA implants accomplish in a medium of physiological pH. The monitoring of the mass loss, the chromatograms, and the M_w values along the time proved to be useful for evaluating the erosion/degradation processes, as well as for inferring the involved degradation mechanisms. Since our raw PLGA presented a bimodal chromatogram, we were unable to associate the core and surface degradation of the implant with a characteristic mode. Instead, we related them with the observed chromatogram behavior, which in turn may be associated with the pH of the surrounding medium.

The Imp-PBS underwent a time-variant degradation process characterized by two well-differentiated phases: 1) an autocatalytic bulk degradation phase occurring before the implant breakage (at around the second week); and 2) an uncatalyzed surface degradation phase occurring after the implant breakage. Degradation in both phases was independently justified by a monotonically-decreasing profile of the remaining polymer mass. The use of PLGA microspheres degraded in the two SS and PBS media were useful to study the degradation of different pH-affected areas of large devices and to deconvolve the degradation phenomena. The methodology based on the analysis of auxiliary microspheres systems appears as a promising tool which may enable a better understanding of the degradation process of other large PLGA systems.

Acknowledgments

In memory of Dr. Ricardo J.A. Grau and Dra. María Inés Cabrera, who passed away while this paper was in preparation. The authors are grateful for the financial support received from the following Argentine institutions: Agencia Nacional de Promoción Científica y Tecnológica (ANPCYT), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), and Universidad Nacional del Litoral (UNL).

References

1. Rajeev A J (2000) The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. Biomaterials 21 (23):2475-2490. doi:10.1016/s0142-9612(00)00115-0

2. Fredenberg S, Wahlgren M, Reslow M, Axelsson A (2011) The mechanisms of drug release in poly(lactic-co-glycolic acid)-based drug delivery systems—A review. Int J Pharm 415 (1-2):34-52. doi:10.1016/j.ijpharm.2011.05.049

3. Hatefi A, Amsden B (2002) Biodegradable injectable in situ forming drug delivery systems. J Control Release 80 (1-3):9-28. doi:10.1016/s0168-3659(02)00008-1

4. Packhaeuser CB, Schnieders J, Oster CG, Kissel T (2004) In situ forming parenteral drug delivery systems: an overview. Eur J Pharm Biopharm 58 (2):445-455. doi:10.1016/j.ejpb.2004.03.003

5. Jain GK, Pathan SA, Akhter S, Ahmad N, Jain N, Talegaonkar S, Khar RK, Ahmad FJ (2010) Mechanistic study of hydrolytic erosion and drug release behaviour of PLGA nanoparticles: Influence of chitosan. Polym Degrad Stabil 95 (12):2360-2366. doi:10.1016/j.polymdegradstab.2010.08.015

6. Göpferich A (1997) Mechanisms of polymer degradation and elimination. In: Domb AJ, Kost J, Wiseman DM (eds) Handbook of Biodegradable Polymers. Harwood Academic Publishers, Amsterdam, pp 451–471

7. Göpferich A (1996) Mechanisms of polymer degradation and erosion. Biomaterials 17 (2):103-114. doi:10.1016/0142-9612(96)85755-3

8. Li SM, Garreau H, Vert M (1990) Structure-property relationships in the case of the degradation of massive aliphatic poly-(α-hydroxy acids) in aqueous media. J Mater Sci: Mater Med 1 (3):123-130. doi:10.1007/bf00700871

9. Tamada JA, Langer R (1993) Erosion kinetics of hydrolytically degradable polymers. Proc Natl Acad Sci U S A 90 (2):552-556

10. Yoshioka T, Kawazoe N, Tateishi T, Chen G (2008) In vitro evaluation of biodegradation of poly(lactic-co-glycolic acid) sponges. Biomaterials 29 (24–25):3438-3443. doi:10.1016/j.biomaterials.2008.04.011

11. Grizzi I, Garreau H, Li S, Vert M (1995) Hydrolytic degradation of devices based on poly(dl-lactic acid) size-dependence. Biomaterials 16 (4):305-311

12. Chen Y, Zhou S, Li Q (2011) Mathematical modeling of degradation for bulk-erosive polymers: Applications in tissue engineering scaffolds and drug delivery systems. Acta Biomater 7 (3):1140-1149

13. Krebs MD, Sutter KA, Lin ASP, Guldberg RE, Alsberg E (2009) Injectable poly(lactic-co-glycolic) acid scaffolds with in situ pore formation for tissue engineering. Acta Biomater 5 (8):2847-2859. doi:10.1016/j.actbio.2009.04.035

14. Dånmark S, Finne-Wistrand A, Schander K, Hakkarainen M, Arvidson K, Mustafa K, Albertsson AC (2011) In vitro and in vivo degradation profile of aliphatic polyesters subjected to electron beam sterilization. Acta Biomater 7 (5):2035-2046. doi:10.1016/j.actbio.2011.02.011

15. Körber M (2010) PLGA erosion: Solubility- or diffusion-controlled? Pharmaceut Res 27 (11):2414-2420

16. Schliecker G, Schmidt C, Fuchs S, Wombacher R, Kissel T (2003) Hydrolytic degradation of poly(lactide-co-glycolide) films: effect of oligomers on degradation rate and crystallinity. Int J Pharm 266 (1-2):39-49. doi:10.1016/s0378-5173(03)00379-x

17. Wang L, Venkatraman S, Kleiner L (2004) Drug release from injectable depots: two different in vitro mechanisms. J Control Release 99 (2):207-216. doi:10.1016/j.jconrel.2004.06.021

18. Graham PD, Brodbeck KJ, McHugh AJ (1999) Phase inversion dynamics of PLGA solutions related to drug delivery. J Control Release 58 (2):233-245

19. Dunn RL, English, James P., Cowsar, Donald R., Vanderbilt, David P. (1990) Biodegradable in-situ forming implants and methods of producing the same. US Patent Patent 5990194.

20. Sah H (1997) Microencapsulation techniques using ethyl acetate as a dispersed solvent: effects of its extraction rate on the characteristics of PLGA microspheres. J Control Release 47 (3):233-245. doi:http://dx.doi.org/10.1016/S0168-3659(97)01647-7

21. Mohd-Adnan A-F, Nishida H, Shirai Y (2008) Evaluation of kinetics parameters for poly(l-lactic acid) hydrolysis under high-pressure steam. Polym Degrad Stabil 93 (6):1053-1058. doi:10.1016/j.polymdegradstab.2008.03.022

22. Sadao Mori HGB (1999) Size exclusion chromatography. Springer Laboratory, Springer Lab Manuals Series, Laboratory Series, 1st edn. Springer, Germany

23. Meira GR, Vega JR, Yossen MM (2004) Gel Permeation and Size Exclusion Chromatography. In: Cazes J (ed) Ewing's Analytical Instrumentation Handbook. 3rd edn. Marcel Dekker Inc., New York, pp 827-869

24. Kenley RA, Lee MO, Mahoney TR, Sanders LM (1987) Poly(lactide-co-glycolide) decomposition kinetics in vivo and in vitro. Macromolecules 20 (10):2398-2403

25. Chiu LK, Chiu WJ, Cheng YL (1995) Effects of polymer degradation on drug released - mechanistic study of morphology and transport properties in 50:50 poly(dl-lactide-co-glycolide). Int J Pharm 126 (1-2):169-178

26. Göpferich A (1997) Polymer bulk erosion. Macromolecules 30 (9):2598-2604

27. Vey E, Rodger C, Booth J, Claybourn M, Miller AF, Saiani A (2011) Degradation kinetics of poly(lactic-co-glycolic) acid block copolymer cast films in phosphate buffer solution as revealed by infrared and Raman spectroscopies. Polym Degrad Stabil 96 (10):1882-1889. doi:10.1016/j.polymdegradstab.2011.07.011

28. Mobedi H, Nekoomanesh M, Orafaei H, Mivehchi H (2006) Studying the Degradation of Poly(L-lactide) in Presence of Magnesium Hydroxide. Iran Polym J 15 (1):31-39

29. Zolnik BS, Burgess DJ (2007) Effect of acidic pH on PLGA microsphere degradation and release. J Control Release 122 (3):338-344. doi:10.1016/j.jconrel.2007.05.034

30. Park TG (1994) Degradation of poly(d,l-lactic acid) microspheres: effect of molecular weight. J Control Release 30 (2):161-173. doi:http://dx.doi.org/10.1016/0168-3659(94)90263-1

31. Dong WY, Körber M, López Esguerra V, Bodmeier R (2006) Stability of poly(d,l-lactide-co-glycolide) and leuprolide acetate in in-situ forming drug delivery systems. J Control Release 115 (2):158-167. doi:10.1016/j.jconrel.2006.07.013

32. Gasparini G, Holdich RG, Kosvintsev SR (2010) PLGA particle production for water-soluble drug encapsulation: Degradation and release behaviour. Colloid Surface B 75 (2):557-564

33. Blasi P, D'Souza SS, Selmin F, DeLuca PP (2005) Plasticizing effect of water on poly(lactide-co-glycolide). J Control Release 108 (1):1-9

34. Yoo JY, Kim JM, Seo KS, Jeong YK, Lee HB, Khang G (2005) Characterization of degradation behavior for PLGA in various pH condition by simple liquid chromatography method. Biomed Mater Eng 15 (4):279-288

35. Lam CXF, Savalani MM, Teoh S-H, Hutmacher DW (2008) Dynamics of in vitro polymer degradation of polycaprolactone-based scaffolds: accelerated versus simulated physiological conditions. Biomed Mater 3 (3):034108

Figure Legends

Fig. 1. SEM micrographs of (a) PLGA implant formed by phase inversion after 1 day of incubation in PBS and (b) microspheres before degradation.

Fig. 2. pH change of the incubation media along the degradation time for the three investigated systems: (a) Ms-SS; (b) Imp-PBS; (c) Ms-PBS.

Fig. 3. SEM micrographs of PLGA in situ formed implant at 3, 12, 22 and 33 days of incubation. The areas indicated by C and W correspond to the core and the wall of the implant, respectively. The black arrow points to the dialysis bag.

Fig. 4. Evolution of the percentage remaining mass (*RM*) along the degradation time, for the three investigated systems. Error bars correspond to standard deviations of the data.

Fig. 5. Fitting of Eq. (4) to the experimental *RM* data, for the three investigated systems.

Fig. 6. Evolution of the chromatograms normalized to the remaining mass for the three investigated systems. The dotted line (0d) corresponds to the raw PLGA.

Fig. 7. Logarithmic peak molecular weight ($\log M_p$) for the three investigated systems as a function of the degradation time. The symbols (⋯◇⋯) and (⋯✦⋯) correspond to high and low modes, respectively. Filled symbols correspond to $\log M_p$ of the raw PLGA.

Fig. 8. Evolution of the weight-average molecular weights (M_w) during the degradation of the three investigated systems. Fitting of the experimental data with the model of Eq. (5). Autocatalyzed degradation is indicated by doted line; uncatalyzed degradation is indicated by continuous line. The symbol✦ corresponds to the M_w of the raw PLGA.

Fig. 9. Schematic visualization of the different degradation processes along the time (higher color density represents a lower degradation degree). (a) Typical bulk and surface erosion processes, and the proposed degradation/erosion process for implants in PBS; (b) Time evolution of chromatograms and M_w of the implant degraded in a physiological medium of maintained pH (Imp-PBS). The time t_{ib} represents the time of the implant breakage.

Table
Click here to download Table: Table 1.doc

1

Table 1

Erosion parameters obtained by fitting Eq. (4) to the measured *RM*, for the three investigated systems[a]

	Ms-SS[b]	**Imp-PBS**	**Ms-PBS**
Experimental points (n)	11	14	10
t_{lag} (day)	8	0	0
k (day^{-1})	0.124	0.04	0.03
R^2	0.976	0.993	0.990

[a] The goodness of fit was done using the standard correlation coefficient R^2; [b] Fit done from day 8 of degradation onward.

2

Table
Click here to download Table: Table 2.doc

1

Table 2

Degradation parameters obtained by fitting Eq. (5) to the measured M_w, for the three investigated systems[a]

	Ms-SS[b]	Imp-PBS		Ms-PBS
		autocatalyzed degradation[c]	uncatalyzed degradation[d]	
Experimental points (n)	7	6	11	10
λ (day^{-1})	0.101	0.036	≈0	≈0
A	0.78	0.86	0.46	1.02

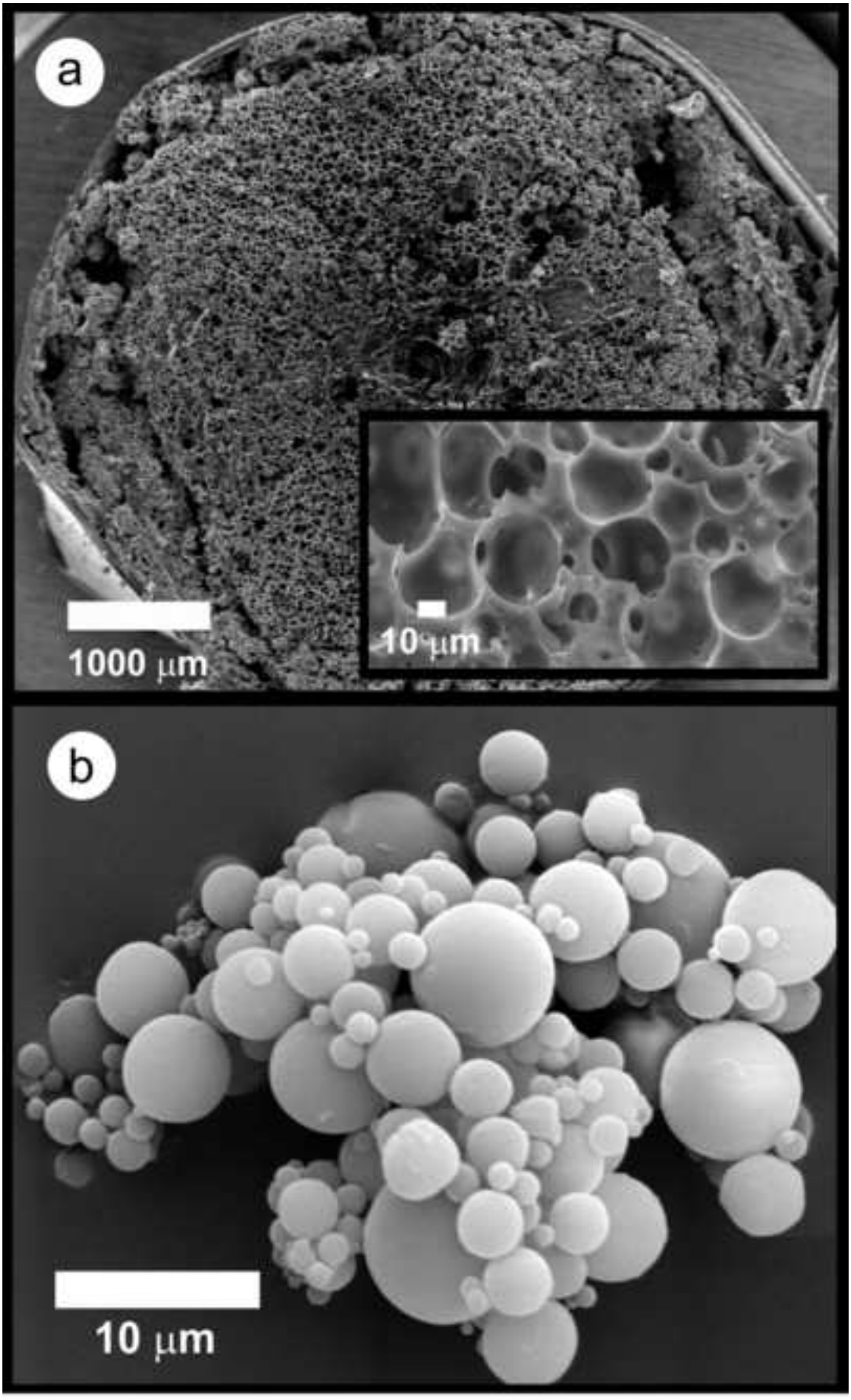
[a] The goodness of fit was done using a Chi-square test, with a significant level of α=0.01; [b] Fit done up to day 14 of degradation; [c] Fit done up to day 16 of degradation; [d] Fit done since day 18 of degradation.

2

3

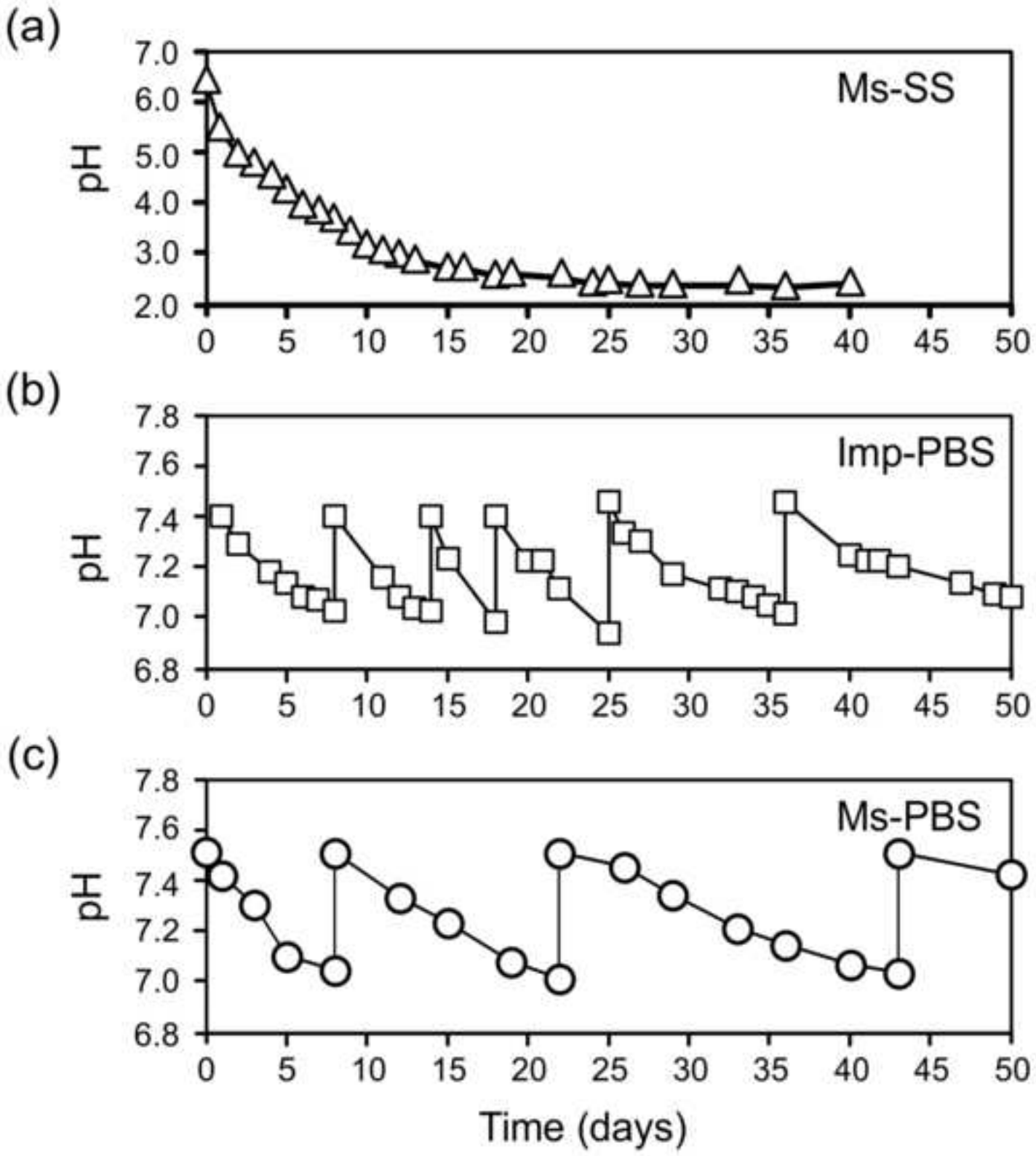
4

Figure1
Click here to download Figure: Figure 1.tif



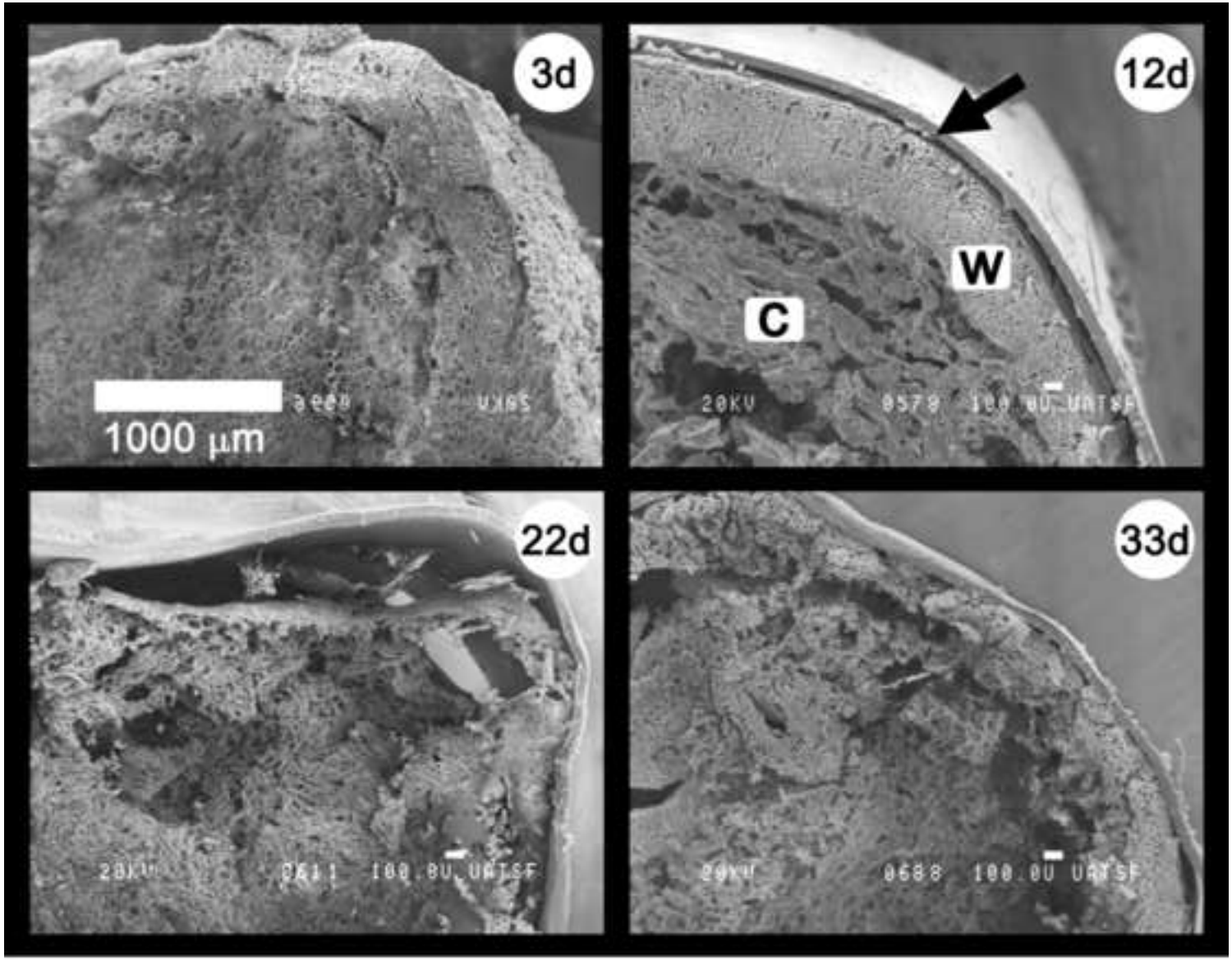
Boimvaser et al.
Figure 1

Figure2
Click here to download Figure: Figure 2.tif



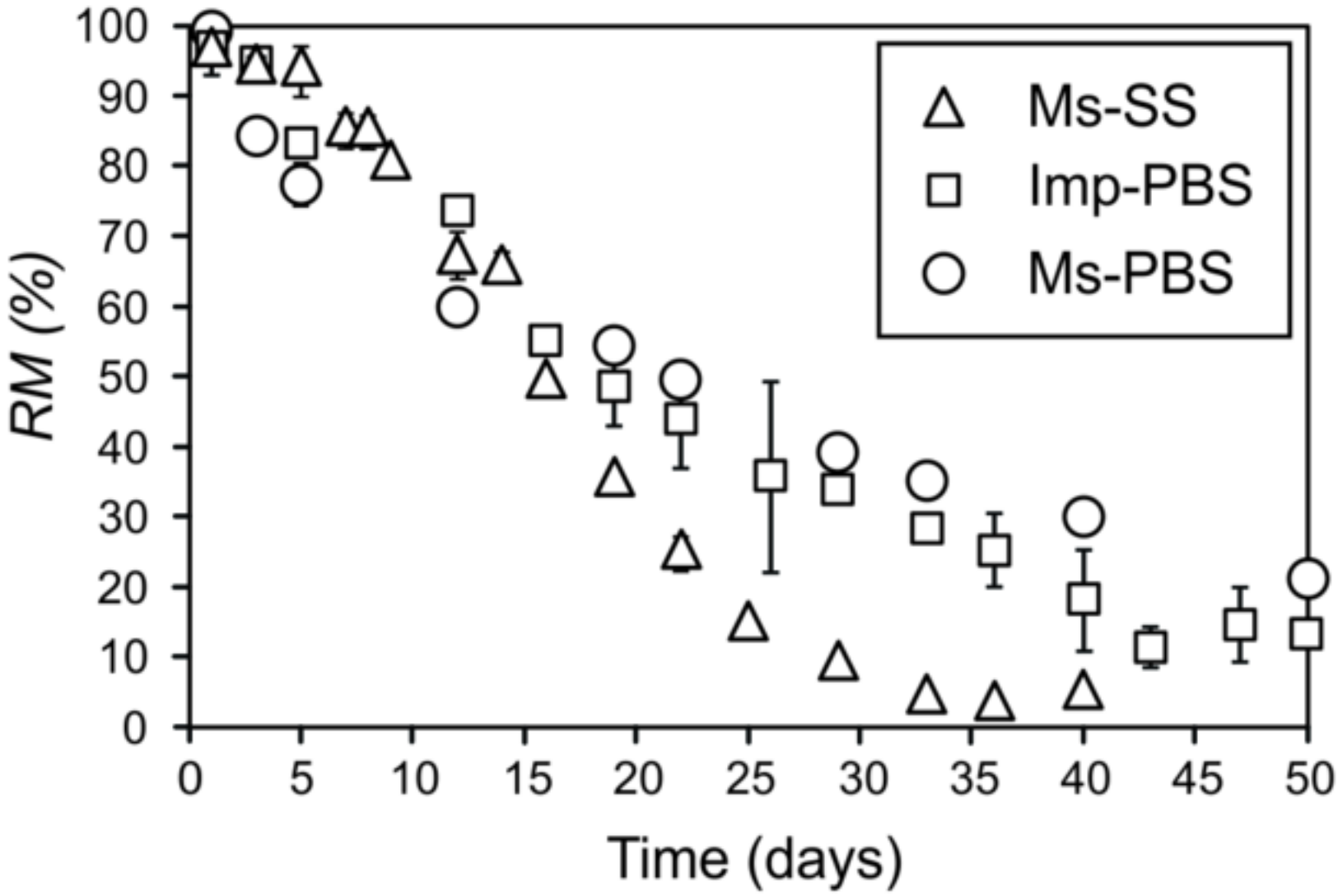
Boimvaser et al.
Figure 2

Figure3
Click here to download Figure: Figure 3.tif



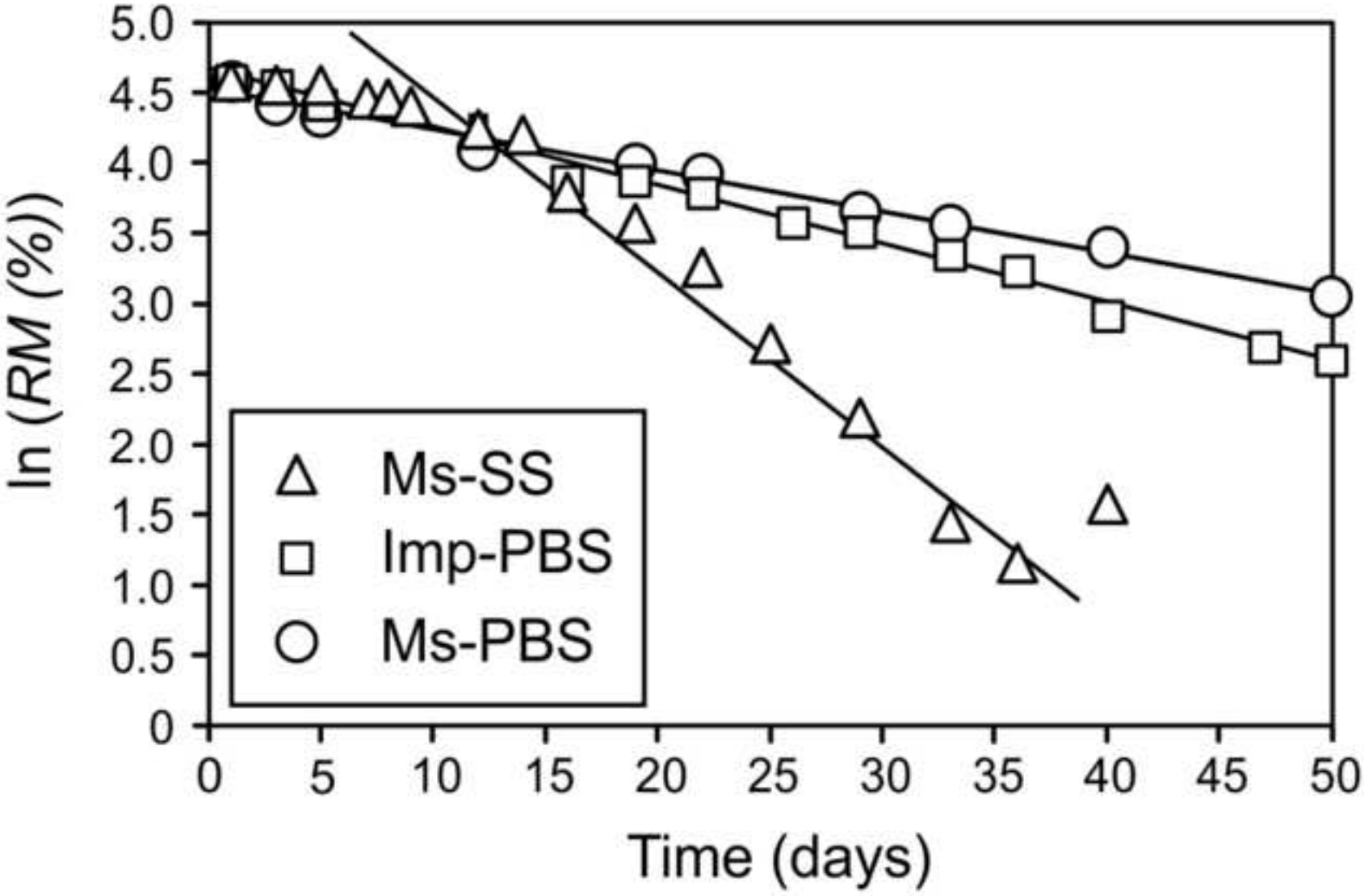
Boimvaser et al.
Figure 3

Figure4
Click here to download Figure: Figure 4.tif



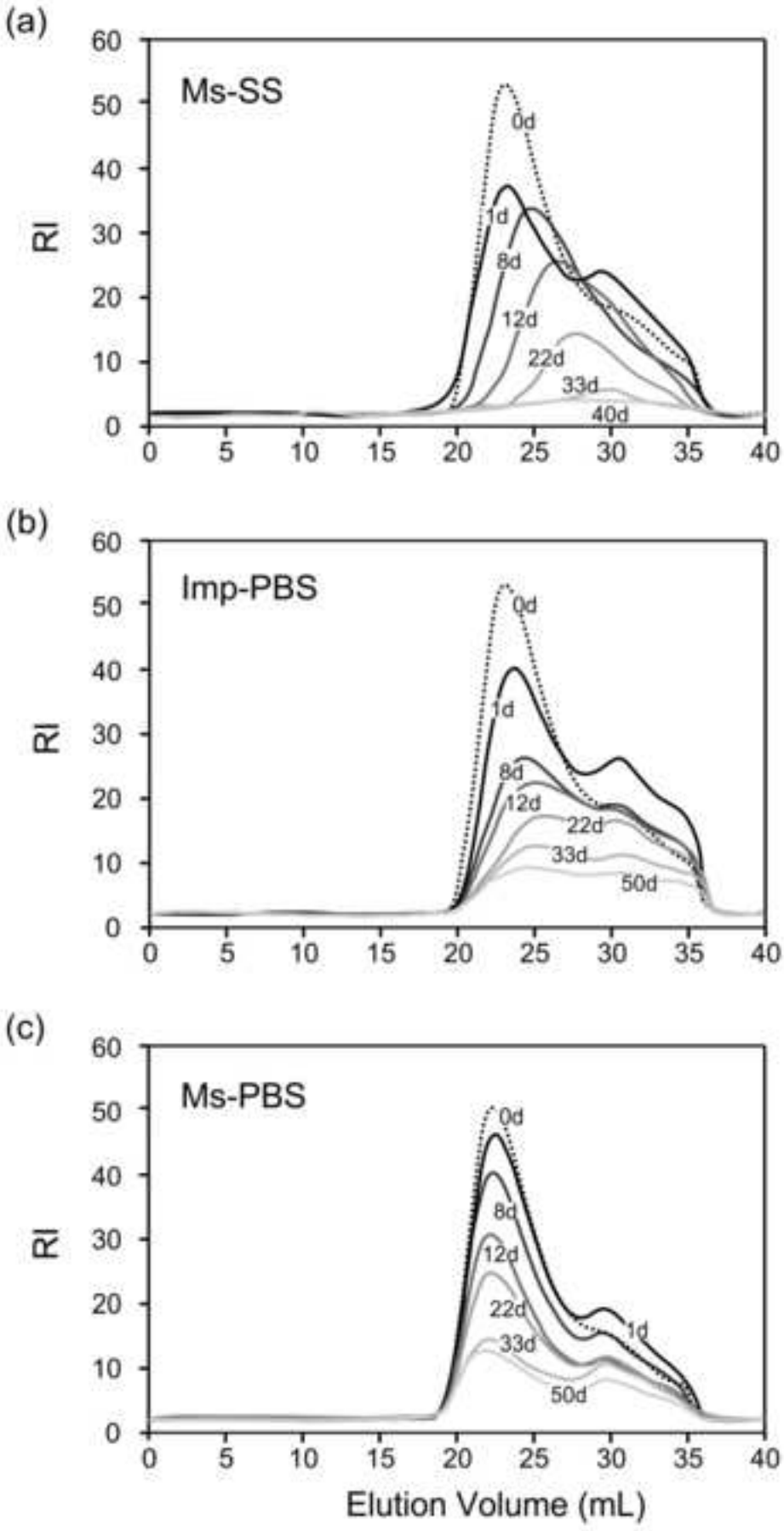
Boimvaser et al.
Figure 4

Figure5
Click here to download Figure: Figure 5.tif



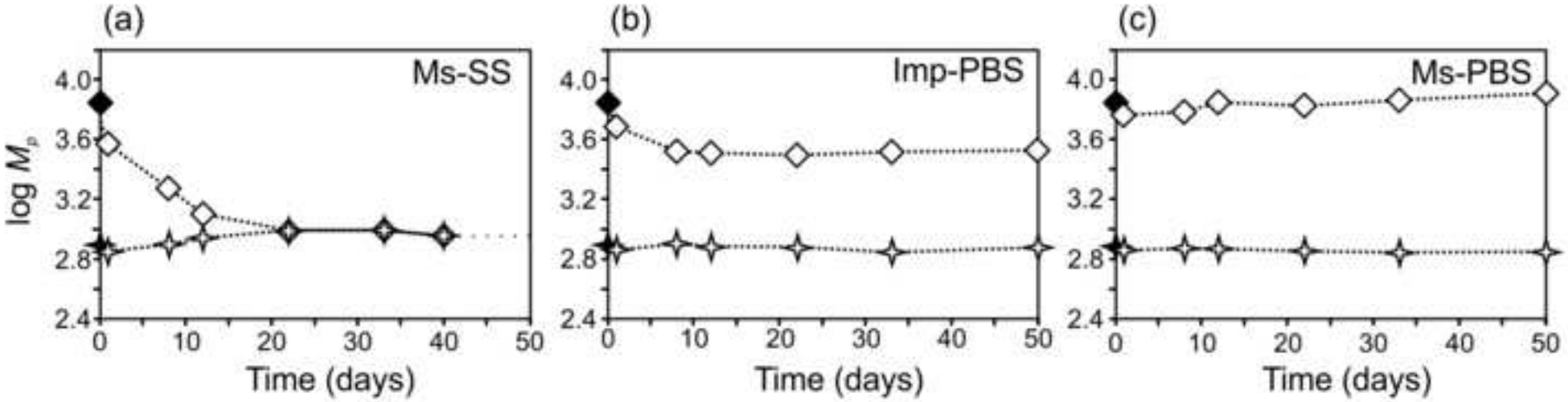
Boimvaser et al.
Figure 5

Figure6
Click here to download Figure: Figure 6.tif



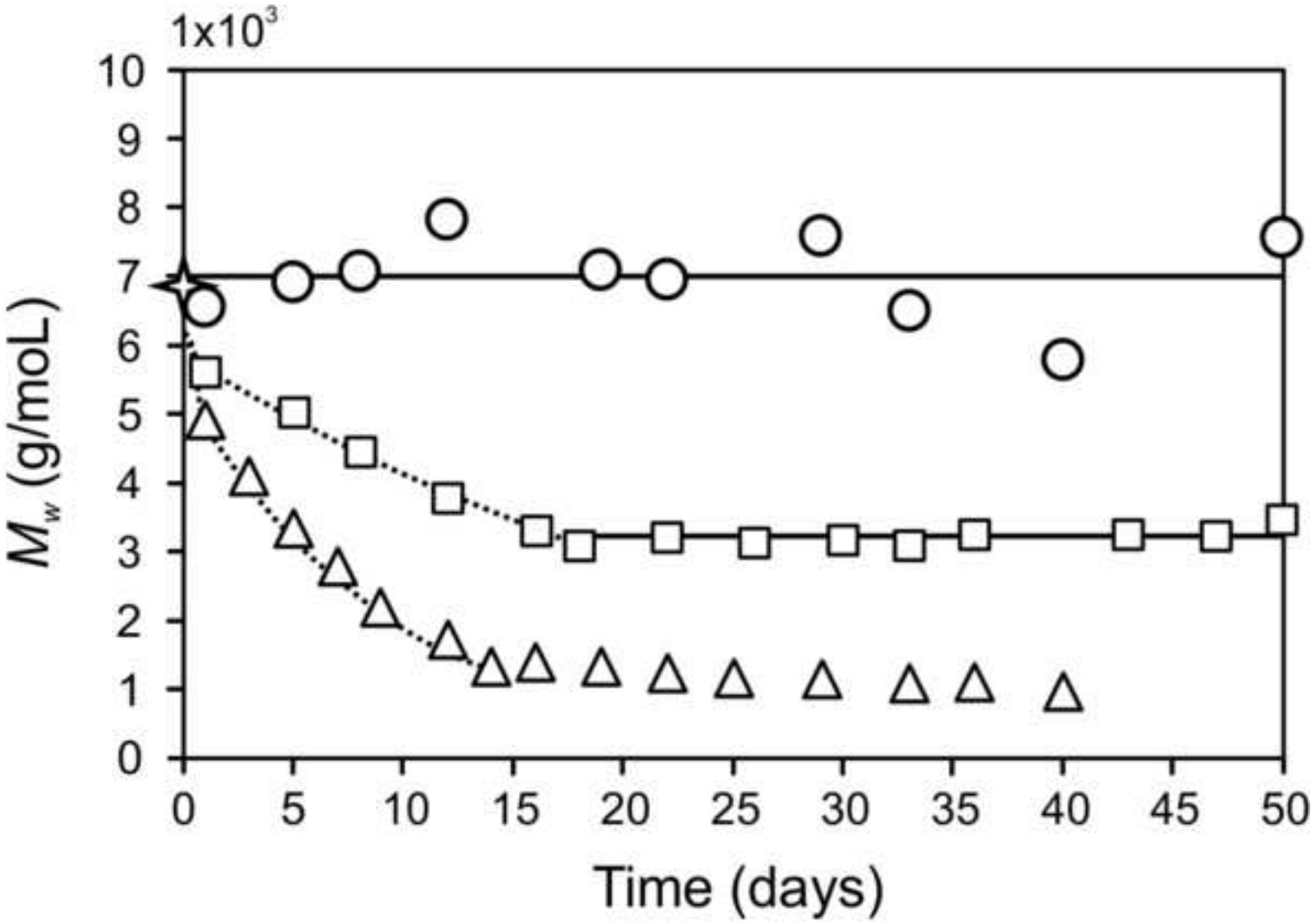
Boimvaser et al.
Figure 6

Figure7
Click here to download Figure: Figure 7.tif



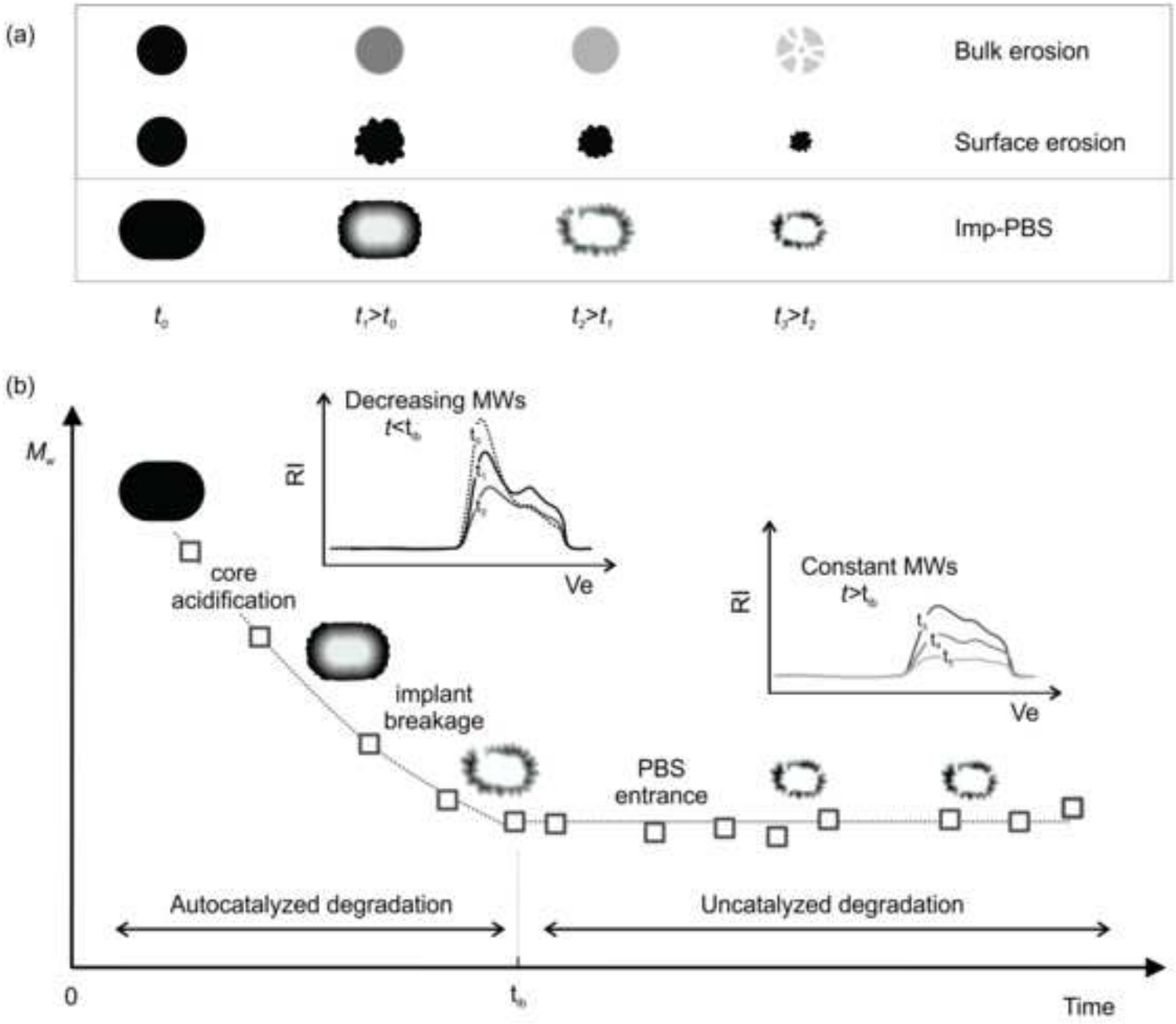
Boimvaser et al.
Figure 7

Figure8
Click here to download Figure: Figure 8.tif



Boimvaser et al.
Figure 8

Figure9
Click here to download Figure: Figure 9.tif



Boimvaser et al. Figure 9